Biomedicine & Pharmacotherapy 86 (2017) 95–101

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Aqueous fraction of *Alstonia boonei* de Wild leaves suppressed inflammatory responses in carrageenan and formaldehyde induced arthritic rats

Omowumi O. Akinnawo, God'swill N. Anyasor*, Odutola Osilesi

Department of Biochemistry, Benjamin S. Carson (Snr.) School of Medicine, Babcock University, Ilishan-Remo, Ogun State, PMB, 21244 Ikeja, Nigeria

ARTICLE INFO

Article history:
Received 28 October 2016
Received in revised form 23 November 2016
Accepted 30 November 2016

Keywords:
Alstonia boonei
Anti-Inflammation
Antioxidant
Phytochemicals

ABSTRACT

Alstonia boonie de Wild is an ethnomedical plant used as therapy against inflammatory disorders. This study evaluated the most active anti-inflammatory and anti-oxidant fraction of *A. boonei* leaves using *in vitro* and *in vivo* models. Quantitative phytochemical analysis, anti-protein denaturation and hypotonicity-induced hemolysis of human red blood cell membrane (HRBC), radical scavenging activity assays, carrageenan and formaldehyde-induced inflammation models were carried out. Results showed that aqueous and ethyl acetate fractions of 70% methanol extract of *A. boonie* leaves contained high quantities of total phenolic and flavonoid compounds compared with hexane and butanol fractions. Aqueous fraction of *A. boonie* leaves significantly ($P < 0.05$) inhibited heat-induced protein denaturation, stabilized hypotonicity-induced hemolysis of HRBC, scavenged DPPH, $NO^{\bullet}$ and H_2O_2 radicals in a concentration-dependent manner compared with other fractions in vitro. In addition, orally administered 50–250–mg/kg body weight (b.w.) aqueous fraction of *A. boonei* leaves suppressed carrageenan-induced rat paw edema thickness by 74.32%, 79.22% and 89.86% respectively at 6th h in a dose-dependent manner comparable with animals treated with standard diclofenac sodium (88.69%) *in vivo*. Furthermore, investigation of formaldehyde-induced inflammation in rats showed that 50–250 mg/kg b.w. aqueous fraction of *A. boonei* reduced plasma alanine aminotransferase and aspartate aminotransferase activities. Aqueous fraction of *A. boonei* also suppressed eosinophils, monocytes and basophils, total white blood cell, total platelet, neutrophil and lymphocyte counts and modulated plasma lipid profile compared with control group. Aqueous fraction of *A. boonei* leaves exhibited substantial active anti-inflammatory and antioxidant activities. Hence, an aqueous fraction of *A. boonei* leaves could be channeled towards pharmaceutical drug development. In addition, this study provided scientific insight to account for the traditional use of *A. boonei* leaves in ethnomedical practice.

1. Introduction

Medicinal plants are known to contain important bioactive compounds that could be harnessed for the discovery and development of novel therapeutic drugs. These drugs of plant origin could possess therapeutic advantage against inflammatory and oxidative stress related diseases with minimal side effects than conventional drugs [1].

Inflammation is considered primarily as a physiologic defence mechanism which protects the body system against infections, toxins, allergens or other noxious stimuli [2]. However, persistent inflammation may act as an etiologic factor for many chronic debilitating musculoskeletal disorders [3]. In addition, upregulation of reactive oxygen and nitrogen species, prostaglandins, histamines, bradykinins, leukotrienes and polymorphonuclear

Abbreviation: ALT, alanine aminotransferase; AST, aspartate amino transferase; BUHREC, babcock university health, research ethics committee; COX-2, cyclooxygenase 2; DPPH, 1,1-deiphenyl 2-picryl hydrazyl; HRBC, human red blood cell membrane; EMB, eosinophils, monocytes and basophils; GC–MS, gas chromatography mass spectrometry; HDL, high density lipoprotein; IC_{50}, fifty percent inhibitory concentration; LDL, low density lipoprotein; NRC, national research council; NSAIDs, non-steroidal anti-inflammatory drugs; OECD, organization for economic cooperation and development; RT, retention time; SEM, standard error of mean; TP, total protein; VLDL, very low density lipoprotein; WBC, white blood cell.

* Corresponding author.
E-mail addresses: wumibukola@gmail.com (O.O. Akinnawo), anyasorg@babcock.edu.ng (G.N. Anyasor), odutola.osilesi@gmail.com (O. Osilesi).

http://dx.doi.org/10.1016/j.biopha.2016.11.145
0753-3322/

cells had been implicated in the pathogenesis of several inflammatory diseases including rheumatoid arthritis, Alzheimer's, Parkinson, arteriosclerosis and aging [4].

Currently, disorders associated with inflammation are managed through the implementation of various intervention strategies aimed at suppressing pro-inflammatory mediators. However, application of steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) by patients with inflammatory disorders have been associated with severe adverse effects. Hence, there is a need to discover and develop an alternative anti-inflammatory drug of plant origin with minimal or no side effects.

Numerous scientific investigations have verified that herbal remedies elicit maximum therapeutic benefits with minimum side effects [1]. In addition, medicinal plants are generally more acceptable to ethnomedical practitioners when compared to the expensive synthetic medicines and majority of the world population especially in developing countries depends on herbal medicines to meet their health requirements since synthetic medicine are usually faced with the challenge of adulteration which influences its efficacy [5].

Alstonia boonei de Wild of the family Apocynaceae, commonly called "devil tree" consist of about 40–60 species widely used ethno-medically for the treatment of several inflammatory-associated disorders [6]. It is found mostly in tropical and subtropical Africa, Southeast Asia, Central America and Australia [7]. *A. boonei* is a large deciduous evergreen tree with a height of about 45 m and diameter of 1.2 m. In the South-Western Nigeria it is called "Awun", South-Eastern Nigerian calls it "Egbu-ora" and "Ukpukunu" by South-Central Nigeria [6].

A. boonie leaves and stem bark extracts have been used in ethnomedical practice as therapy for the treatment of arthritis and rheumatism [8]. The stem bark of *A. boonei* has been reported to possess potent neuroleptic and anxiolytic properties in mice [9]. It also contains minerals like calcium, phosphorous, iron, sodium, potassium and magnesium and phytochemicals including alkaloids, tannins, saponins, flavonoids, cardiac glycoside and vitamin C [6]. However, there is little or no scientific validation of the use of *A. boonei* leaves extract in the management of inflammatory disorders. Therefore, this study was designed to evaluate the anti-inflammatory and antioxidant fraction of *Alstonia boonei* leaves using *in vitro* and *in vivo* models with the objective to proffer scientific explanation to the ethnomedical use of this plant.

2. Materials and methods

2.1. Plant collection and identification

Fresh leaves of *A. boonei* were collected from a farm in Ondo State, Nigeria. It was authenticated by Professor E.B. Esan, Plant Breeding and Biotechnology, Department of Basic Sciences and voucher sample with number FHI 107254 has been deposited at the Forestry Research Institute of Nigeria, Ibadan, Nigeria.

2.2. Animals

Sixty male albino rats (Wistar strains), weighing between 150 and 250 g were purchased from an inbred colony, Babcock University Animal Facility. Animals were acclimatized at $28 \pm 5\,^{\circ}C$ with a $55 \pm 5\%$ relative humidity in a standard wire mesh wooden cage under 12 h light/dark cycle for 14 d prior to experimentation. Animals were provided with commercial pelleted rat chow and water. All animal experimental protocols were in conformity with the National Institute of Health/National Research Council (NRC) Regulations on Laboratory Animal Care and Use Guidelines [10]. Institutional ethical approval with identification number BUHREC065/14 was obtained from the Babcock University Health, Research Ethics Committee (BUHREC). Acute toxicity testing was in line with the median lethal dose of the test fraction in rats according to the Organization for Economic Cooperation and Development (OECD) guidelines 425 [11].

2.3. Preparation of plant extract

A. boonei leaves were thoroughly washed to remove debris, oven-dried at 35 °C and subsequently pulverized using Warring blender. Pulverized *A. boonei* leaves (300 g) were soaked with 1800 ml 70% methanol in ratio 1:8 (w/v) and mixed intermittently for 48 h. Subsequently, the suspension was filtered using Whatman No. 1 filter paper. The obtained filtrate was concentrated using a rotary evaporator (RE52-3 model, LIDA Instrument) at 45 °C. The concentrate was immediately reconstituted in distilled water in a ratio of 1:2 (concentrate: distilled water) and further partitioned using successive solvent partitioning method as follows: hexane, butanol, ethyl acetate and the remaining fraction was considered as an aqueous fraction. The different solvent fractions were concentrated using rotary evaporator at 40–45 °C and stored in a refrigerator until further use.

2.4. Quantitative phytochemical analysis

Total phenolic content was determined according to the method of Singleton et al. [12]. Flavonoid content was determined using the complex aluminum chloride method as described by Ordonez et al. [13]. Tannin content was determined according to the modified vanillin-HCl methanol method as described by Noha et al. [14]. Percentage saponins and alkaloids were determined using gravimetric methods as described by Okwu and Josiah [15] and Onyilagba and Islam [16] respectively.

2.5. In vitro *assays for anti-inflammatory and antioxidant determination*

Stabilization of hypotonicity-induced hemolysis of human red blood cell membrane (HRBC) by *A. boonei* fractions was carried out using the method described by Sadique et al. [17]. Anti-thermal induced protein denaturation by the fractions was performed using the method described by Sakat et al. [18] with minor modifications. DPPH (1,1-diphenyl 2-picryl hydrazine) radical scavenging activity was carried out according to the method described by Cox et al. [19]. Determinations of nitric oxide radical and hydrogen peroxide scavenging activities were carried out using the methods adopted by Ebrahimzadeh et al. [20] and Karunakaran and Kumaran [21] respectively.

2.6. Assay for anti-inflammatory activity (in vivo *models)*

2.6.1. Model I: carrageenan-induced paw edema

Effect of *A. boonei* fraction on carrageenan-induced rat paw edema was carried out using the protocol described by Shashank et al. [22]. Thirty rats were randomly assigned into six groups of 5 rats each. Right hind paw thickness of each rat was initially measured using a micrometer screw gauge, this was followed by the oral administration of different doses of *A. boonei* leaves aqueous fraction and diclofenac sodium to experimental animals in line with the study protocol. Rat paw edema was induced by injecting 0.1 ml 1% (w/v) carrageenan suspension in 0.9% normal saline subcutaneously into the subplantar region of the right hind paw, one hour after oral administration of test drugs. The increase in paw thickness was measured for 6 h after carrageenan injection.